

WHAT WE CLAIM IS:

1. A method of generating amplified messenger RNAs with polymerase reaction activity, comprising the steps of:
- a. providing a plurality of messenger RNAs for following steps (b) to (f);
 - b. contacting said messenger RNAs with a plurality of first primer sequences to form a plurality of first-strand complementary DNAs, wherein said first-strand complementary DNAs are generated by reverse transcription of said messenger RNAs with extension of said first primers;
 - c. permitting terminal extension of said first-strand complementary DNAs to form a plurality of polynucleotide-tailed first-strand complementary DNAs, wherein said polynucleotide-tailed first-strand complementary DNAs are tailed by multiple copies of deoxynucleotides;
 - d. incubating denatured said polynucleotide-tailed first-strand complementary DNAs with a plurality of second promoter-containing primers to form a plurality of double-stranded complementary DNAs, wherein said double-stranded complementary DNAs are generated by extension of DNA polymerase activity with said second promoter-containing primers;
 - e. permitting transcription of said double-stranded complementary DNAs to form a plurality of amplified RNAs, wherein said amplified RNAs are generated by extension of RNA polymerase activity through the promoter region of said double-stranded complementary DNAs; and
 - f. contacting said amplified RNAs with said first primer sequences to form a plurality of said polynucleotide-tailed first-strand complementary DNAs,

wherein said polynucleotide-tailed first-strand complementary DNAs are generated by reverse transcription of said amplified RNAs with extension of said first primer-sequences.

2. The method as defined in Claim 1, further comprising repeated steps (d) through (f) at least one time.
3. The method as defined in Claim 1, further comprising the step of cell fixation for preventing intracellular RNA degradation before the step (a).
4. The method as defined in Claim 1, further comprising the step of cloning said double-stranded complementary DNAs into competent vectors after the step (f).
5. The method as defined in Claim 1, further comprising the step of incorporating a plurality of cap-nucleotides into 5'-ends of said amplified messenger RNAs for in vitro translation in the step (e).
6. The method as defined in Claim 5, wherein said cap-nucleotide is selected from the group consisting of P¹-5'-(7-methyl)-guanosine-P³-5'-adenosine-triphosphate, P¹-5'-(7-methyl)-guanosine-P³-5'-guanosine-triphosphate, P¹-5'-(7-methyl)-guanosine-P³-5'-cytidine-triphosphate and P¹-5'-(7-methyl)-guanosine-P³-5'-uridine-triphosphate.
7. The method as defined in Claim 1, wherein said reverse transcription is an enzyme activity selected from the group consisting of AMV, M-MuLV, HIV-1 reverse transcriptase and Tth-like DNA polymerases with reverse transcription activity.
8. The method as defined in Claim 7, wherein said enzyme activity is performed at temperature ranged from about 37°C to about 80°C.

9. The method as defined in Claim 1, wherein said first primer sequences are complementary to the tails of said messenger RNAs.
10. The method as defined in Claim 9, wherein said first primer sequences are coupled to an RNA polymerase promoter and contain about eight to about thirty copies of deoxythymidylates.
11. The method as defined in Claim 1, wherein said denatured polynucleotide-tailed first-strand complementary DNAs are formed at temperature ranged from about 90°C to about 100°C.
12. The method as defined in Claim 1, wherein said DNA polymerase activity is an enzyme activity selected from the group consisting of E. coli DNA polymerase 1, Klenow fragment of E. coli DNA polymerase 1, T4 DNA polymerase, Taq DNA polymerase, Pwo DNA polymerase, Pfu DNA polymerase and Tth-like DNA polymerases, C. therm. polymerase.
13. The method as defined in Claim 12, wherein said DNA polymerase activity is achieved by Tth-like DNA polymerase with reverse transcriptase activity.
14. The method as defined in Claim 12, wherein said DNA polymerase activity is performed at temperature ranged from about 35°C to about 85°C.
15. The method as defined in Claim 1, wherein said second promoter-containing primers are oligonucleotide sequences complementary to the polynucleotide tails of said polynucleotide-tailed first-strand complementary DNAs and also coupled to an RNA polymerase promoter for transcription activity in the step (e).
16. The method as defined in Claim 15, wherein said RNA polymerase promoter is selected from the group consisting of T3, T7, SP6 and M13 RNA polymerase promoter.

Sub B7
17. The method as defined in Claim 1, wherein said transcription is an RNA polymerase activity selected from the group consisting of T3, T7, SP6 and M13 RNA polymerase.

18. The method as defined in Claim 17, wherein said RNA polymerase activity is performed at temperature ranged from about 35°C to about 85°C.

Sub B
19. The method as defined in Claim 18, wherein said RNA polymerase activity is performed at about 37°C.

Sub B8
20. The method as defined in Claim 1, wherein said polynucleotide-tailed first-strand complementary DNAs are tailed by terminal extension activity selected from the group consisting of terminal transferase and M-MuLV reverse transcriptase with multiple copies of same kind of deoxynucleotides.

21. The method as defined in Claim 20, wherein said same deoxynucleotide is selected from the group consisting of deoxyguanylate, deoxycytidylate, deoxyadenylate, deoxythymidylate and deoxyuridylate.

22. A method of performing improved messenger RNA amplification, comprising the steps of:

- Sub. Q4
- a. providing a plurality of messenger RNAs for following steps (b) to (f);
 - b. generating a plurality of polynucleotide-ended complementary DNAs from said messenger RNAs, wherein said polynucleotide-ended complementary DNAs are reverse-transcribed from said messenger RNAs and tailed by multiple deoxynucleotides in the ends;
 - c. permitting denatured said polynucleotide-tailed complementary DNAs to form a plurality of double-stranded complementary DNAs, wherein said double-

stranded complementary DNAs contain a complementary DNA sequence flanked with an RNA polymerase promoter and a polynucleotide-tail; and

- d. incubating said double-stranded complementary DNAs in a plurality of promoter- and tail-dependent extension systems, and thereby providing a plurality of amplified RNAs from said messenger RNAs.

23. The method as defined in Claim 22, further comprising repeated steps (b) through (d) at least one time
24. The method as defined in Claim 22, further comprising the step of cloning the double-stranded complementary DNAs of said amplified messenger RNAs into competent vectors after the step (d).
25. The method as defined in Claim 22, wherein said messenger RNAs are protected by fixation.
26. The method as defined in Claim 22, wherein said messenger RNAs are protected by a plurality of RNase inhibitors.
27. The method as defined in Claim 22, further comprising the step of incorporating a plurality of cap-nucleotides into 5'-ends of said amplified messenger RNAs for in vitro translation in the step (d).
28. The method as defined in Claim 27, wherein said cap-nucleotide is selected from the group consisting of P¹-5'-(7-methyl)-guanosine-P³-5'-adenosine-triphosphate, P¹-5'-(7-methyl)-guanosine-P³-5'-guanosine-triphosphate, P¹-5'-(7-methyl)-guanosine-P³-5'-cytidine-triphosphate and P¹-5'-(7-methyl)-guanosine-P³-5'-uridine-triphosphate.

29. The method as defined in Claim 22, wherein said complementary DNAs are reverse-transcribed by an enzyme activity selected from the group consisting of AMV, M-MuLV, HIV-1 reverse transcriptase and Tth-like DNA polymerases with reverse transcription activity.

30. The method as defined in Claim 22, wherein said RNA polymerase promoter is selected from the group consisting of T3, T7, SP6 and M13 RNA polymerase promoter.

31. The method as defined in Claim 22, wherein said polynucleotide-tailed complementary DNAs are formed by terminal extension activity selected from the group consisting of terminal transferase and M-MuLV reverse transcriptase.

32. The method as defined in Claim 22, wherein said promoter- and primer-dependent extension systems are a plurality of mixed polymerase activities containing RNA polymerase, DNA polymerase and reverse transcriptase.

33. The method as defined in Claim 32, wherein said mixed polymerase activities are selected from the group consisting of T3, T7, SP6, M13 RNA polymerases and Tth-like DNA polymerases with reverse transcriptase activity, C. therm. polymerase.

34. The method as defined in Claim 22, wherein said polynucleotide-tailed complementary DNAs are tailed by terminal extension with multiple copies of same deoxynucleotides.

35. The method as defined in Claim 34, wherein said same deoxynucleotide is selected from the group consisting of deoxyguanylate, deoxycytidylate, deoxyadenylate, deoxythymidylate and deoxyuridylate.

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